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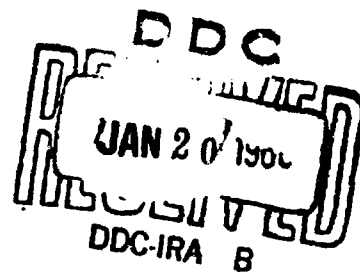
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DIRECT EVIDENCE FOR
THE CATHODIC DEPOLARIZATION THEORY
OF BACTERIAL CORROSION

Warren P. Iverson

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Fort Detrick, Frederick, Maryland

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DIRECT EVIDENCE FOR THE CATHODIC DEPOLARIZATION THEORY
OF BACTERIAL CORROSION

Warren P. Iverson

ABSTRACT

Direct evidence has been obtained to substantiate the cathodic depolarization theory of von Wolzogen Kuhr and van der Vlugt, which involves the removal of hydrogen and reduction of an electron acceptor (benzyl viologen) at the cathode by <u>Desulfovibrio desulfuricans</u> and liberation of iron at the anode. Direct measurement of the cathodic depolarization current has been made and its levels appear to be too low to account for the corrosion of iron in the field by this mechanism. Aluminum alloys can also be cathodically depolarized.

DIRECT EVIDENCE FOR THE CATHODIC DEPOLARIZATION THEORY
OF BACTERIAL CORROSION

In 1934, von Wolzogen Kühr and van der Vlugt proposed a theory for the anaerobic corrosion of iron by bacteria.[1] In brief, the theory states that bacteria, primarily those of the genus _Desulfovibrio_, remove hydrogen that accumulates on the surface of iron as a result of their hydrogenase activity and reduce sulfate, yielding hydrogen sulfide. The electrons removed as a result of hydrogen utilization permit more iron to be dissolved or corroded at the anode. The sulfide and the hydroxyl ions combine with the ferrous ions to form secondary reaction products at the anode (Fig. 1).

$$\text{I} \quad 8H_2O \longrightarrow 8OH^- + 8H^+$$

$$\text{II} \quad 4Fe \longrightarrow 4Fe^{++} + 8e \quad (\text{anode})$$

$$\text{III} \quad 8H^+ + 8e \longrightarrow 8H \quad (\text{cathode})$$

$$\text{IV} \quad SO_4^{--} + 8H \xrightarrow{(\text{bacteria})} S^{--} + 4H_2O \quad (\text{cathodic depolarization})$$

$$\text{V} \quad Fe^{++} + S^{--} \longrightarrow FeS \quad (\text{anode})$$

$$\text{VI} \quad 3Fe^{++} + 6(OH)^- \longrightarrow 3Fe(OH)_2 \quad (\text{anode})$$

$$4Fe + SO_4^{--} + 4H_2O \longrightarrow FeS + 3Fe(OH)_2 + 2(OH)^-$$
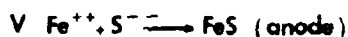
Figure 1. Cathodic Depolarization Theory of
von Wolzogen Kühr and van der Vlugt.

Over the years evidence for and against this theory has accumulated. Much of this earlier evidence has been reviewed by Starkev.[2] More recently, Raifsnider[3] and Scott[4] have presented observations that are not in agreement with this classical theory. Booth and his associates[5-7] using polarization techniques and weight loss measurements vs. hydrogenase act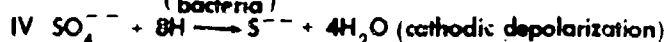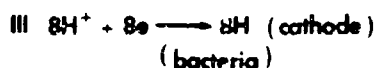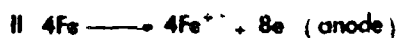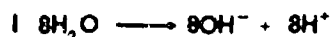ivity, have presented evidence for the theory. These methods, although involving standard techniques, are nevertheless indirect approaches and may be subject to various interpretations. More direct evidence therefore seemed desirable.

Through use of trypticase soy broth (BBL) plus 2% agar it has been possible to culture Desulfovibrio readily and in pure culture on the surface of agar plates under a hydrogen atmosphere. Cells of Desulfovibrio desulfuricans (Mid Continent Strain A), also referred to in this paper as the API (American Petroleum Institute) strain, and possessing hydrogenase activity were grown on the surface of this medium, removed with a bacteriological loop, and placed on a small area on the surface of solidified washed (Noble) agar (2%) containing 0.01 M tris(hydroxymethyl)aminomethane buffer (tris) and benzyl viologen (0.01%). This agar substrate was adjusted to pH 7.0 ±1 with HCl and auto-laved at 15 pounds pressure for 15 minutes. The dye benzyl viologen (BV) is colorless when oxidized and violet when reduced;[8] it was substituted for sulfate as the electron acceptor to avoid the complicating factor of H_2S reaction with the iron and to render the reduction process visible.

Small coupons of polished (emory cloth) 1010 mild steel (8 x 15 mm) were bent into the shape indicated in Figure 2. They were degreased with acetone, sterilized by immersion in ethanol (95%), and the residual alcohol was burned off. After cooling they were placed on the surface of the solidified agar with one end over the area upon which the cells were placed. The plates with the coupons were placed in a jar suitable for evacuation [viz. Brewer's jar, (BBL)] and the atmosphere was replaced with nitrogen (evacuation and replacement three times was usually sufficient).

After about 17 to 24 hours at a temperature of 27±1 C, the plates and coupons were removed. A dark violet area of reduced benzyl viologen was observed in the agar underneath the part of the coupon over the area previously covered with cells (Fig. 3a). Lighter areas of reduced benzyl viologen were observed in the agar underneath both ends of the coupon not in contact with the cells. These lightly reduced areas, probably due to the direct reduction of the dye by the metal, disappeared (oxidized) and left only the heavily reduced area produced by cellular reduction (Fig. 3b). A yellow-brown area could be observed on the surface of the agar surrounding the areas that were in contact with the coupon. This material is apparently an insoluble iron compound, probably Fe_2O_3 or $Fe(OH)_3$, which formed as a result of iron oxidation by small traces of oxygen present in the nitrogen, the agar, or the metal surface.
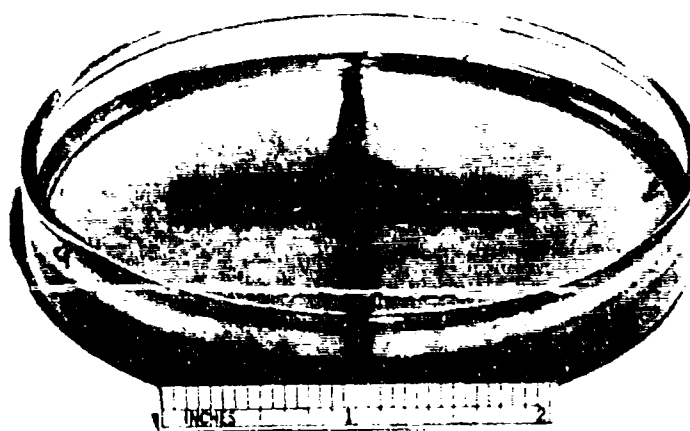
Figure 2. Petri Plate with 1010 Steel Coupon
Resting on Agar Surface.

The iron in the agar was made visible (developed) by adding either potassium thiocyanate (for Fe^{+++}) or potassium ferricyanide (for Fe^{++} or Fe^{+++}) with or without acid (HCl). In Figure 3c equal portions of an aqueous 1% solution of potassium thiocyanate and HCl (10% concentrated v/v) were added to the agar surface. Heavy concentrations of iron were indicated within several minutes where the iron oxide "border" was located. Since this iron was located at the surface the iron thiocyanate complex was washed away by adding more of the reagents. The area of reduced benzyl viologen was still visible. After about twenty minutes only the pink iron thiocyanate complex in the agar that slowly developed (Fe^{++} slowly oxidized to Fe^{+++}) remained visible (Fig. 3d). The reduced benzyl viologen had by that time been oxidized (colorless). Within 30 to 45 minutes, the pink iron thiocyanate complex faded and eventually completely disappeared.
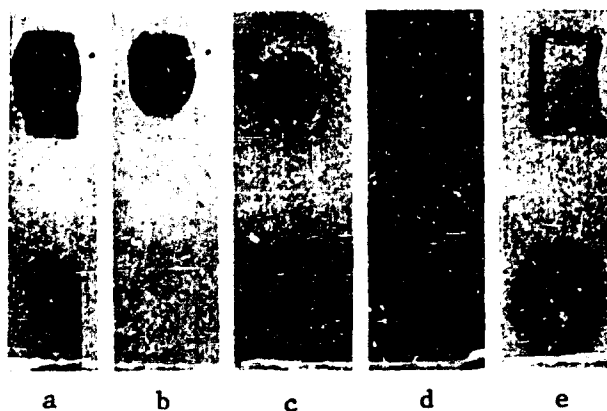
Figure 3. Locations of Reduced Benzyl Viologen and Iron on Agar Plates.

a. Agar surface immediately after removal of steel coupon. Dark area due to reduction of benzyl viologen by _Desulfovibrio_ cells. Lighter area surrounding dark area at cathode and at anode (no cells) due to direct reduction of benzyl viologen by the steel.

b. Same plate 10 minutes later after the benzyl viologen that was reduced directly by the steel has been oxidized (decolorized) by exposure to the air. The dark area of benzyl viologen reduced by the _Desulfovibrio_ cells is still visible. The "borders" of iron oxide are clearly visible.

c. A similar plate to which HCl (10% conc. HCl in H_2O v/v) and potassium thiocyanate (10% w/v) have been added. A heavy iron concentration noted at "border." Area of reduced benzyl viologen still present.

d. Same plate as 3c, 20 minutes after addition of HCl and potassium thiocyanate. The darker areas indicate heaviest iron concentrations at anode and end of cathode, which were not in contact with the cells. The dark area (reduced benzyl viologen) has disappeared (completely oxidized by the oxygen in the atmosphere).

e. Portion of plate that was developed with aqueous potassium ferricyanide (10% w/v), showing a heavy Fe^{++} concentration at anode (no cells) and none at the cathode (area surrounded by black border of masking ink where a heavy concentration of cells was placed). Area of reduced benzyl viologen at cathodic area has been oxidized.

The use of potassium ferricyanide (10% aqueous solution) is much more satisfactory because the color complex does not fade. Potassium ferricyanide reacts with Fe^{++} ions to form a blue precipitate (Turnbull's blue) and with Fe^{+++} to form a green color. It has been found that Fe^{++} ions in small concentrations will also react to form a bluish-green color in the agar, and that this reagent is more sensitive for Fe^{++} ions than Fe^{+++} ions. The potassium ferricyanide solution must be added immediately to the agar surface after removing the plate from the N_2 atmosphere to detect the Fe^{++} ions. After ½ hour or more, the Fe^{++} ions are oxidized to Fe^{+++} ions and cannot be detected by the ferricyanide when the concentrations are low. It is desirable not to add any acid with the ferricyanide because it will dissolve the iron oxide "border" and may confuse the results. A plate developed with ferricyanide is shown (Fig. 3e) that indicates the heavy concentrations of Fe^{++} ions under the coupon not in contact with the cells (anode) and relatively few Fe^{++} ions at the cathode (coupon in contact with the cells).

These results indicate that the _Desulfovibrio_ cells are able to remove hydrogen or electrons from the surface of iron at the cathodic area and use the electrons from the hydrogen to reduce the electron acceptor (the dye benzyl viologen, in this case, which was substituted for sulfate). The deficiency of electrons created by their removal in the formation of hydrogen at the iron surface and the subsequent utilization of hydrogen by the hydrogenase system of the bacterial cells creates a deficiency of electrons at the anode where there are no cells. As a result of this deficiency the equilibrium: $Fe \longrightarrow Fe^{++} + 2e$, is upset and the reaction is displaced to the right, thereby causing more Fe^{++} ions to go into solution (corrode).

Thus, the key step in the theory, namely, the removal of hydrogen or electrons at the cathode and the subsequent dissolution of iron at the anode, appears to be clearly demonstrated.

All of the reactions that are taking place with the steel strip on the agar may be represented in Figure 4. The iron corrodes as a result of three reactions, which take up electrons or act as "electron sinks:" (I) the combination of electrons with oxygen and water to form $(OH)^-$ ions, which react with the Fe^{++} ions and more oxygen to form the oxide "border" effect; (II) the direct uptake of electrons by oxidized BV to form the violet reduced BV; and (III) the reduction of H^+ ions at the iron surface to form molecular or atomic hydrogen, which is then removed and the electrons utilized to reduce the BV.

$$Fe(OH)_2 \quad 2(OH)^-$$

$$\uparrow$$
$$H_2O \qquad I$$
$$+$$
$$\frac{1}{2}O_2$$
$$+ \qquad\qquad II$$
$$Fe \longrightarrow Fe^{++} + \boxed{2e} \quad +2BV \longrightarrow 2BVe$$
$$\text{(oxidized)} \quad \text{(reduced)}$$
$$+ \qquad +$$
$$2H_2O \longrightarrow 2(OH)^- + \quad 2H^+$$
$$\downarrow \qquad\qquad \downarrow$$
$$Fe(OH)_2 \qquad 2H \qquad III$$
$$\downarrow \quad \text{bacteria}$$
$$H_2 + 2BV \longrightarrow 2BVe + 2H^+$$

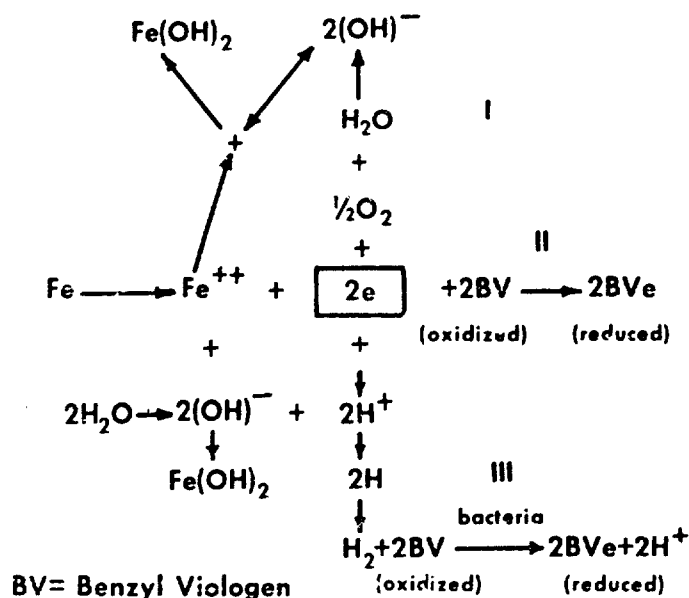BV= Benzyl Viologen $\qquad$ (oxidized) $\qquad$ (reduced)

Figure 4. Corrosion Pathways of Steel Coupon on Benzyl
Viologen, Tris-HCl Buffer Agar. I-reaction
with O_2, II-direct reduction of benzyl viologen,
III-cathodic depolarization (removal of hydrogen)
by bacteria.

The iron that forms the oxide "border" can be distinguished from
reactions II and III by omitting acid in the "development" process.
The iron that forms in reaction II (both anode and cathode) as distin-
guished from reaction III can be detected by using control coupons
(no cells in contact with either end of the coupon). By direct obser-
vation of the color intensity, we note that the total quantity of Fe^{++}
ions evolved from both ends of the control coupon in reaction II is
much less than the total amount of iron evolved at the anode in
reaction III.

Since there was obviously a flow of electrons from the anode to the cathode, direct measurement of this cathodic depolarization current seemed possible. Two electrodes were made from one of the coupons and secured by a plastic holder (Fig. 5) fitted over a standard plastic petri dish bottom (5 cm diameter) so that the electrodes could be dropped and secured on the surface of agar in the petri dish bottom. The surface area of each electrode was approximately 1.1 cm^2.

After the two electrodes were dropped and secured on the agar surface, they were placed in a Brewer's jar and connected by leads that passed through a rubber stopper in the jar top. The jar was evacuated and refilled three times with N_2.

With the tris-HCl buffer benzyl viologen agar described earlier and with the electrodes 1 cm apart (end to end) on the agar surface, the initial internal resistance of the cell was 15,000 ohms. By connecting the leads to a vacuum tube voltmeter that employs a light beam chopper and a-c amplifier (Hewlett-Packard, Model 412A), the current output could be measured and also recorded with an Esterline-Angus recording ammeter Model AW connected to the output of the voltmeter.

It was found that if the electrodes were embedded or encased in plastic (lucite) so that only the electrode surface in contact with the agar was exposed, little if any current would flow without any detectable fluctuations for a period of several days.

By placing a very large quantity of cells (entire surface growth of 3-day-old cells from TSB + agar plate, incubated at 27±1 C) of D. desulfuricans API strain on the surface of solidified tris-HCl buffer benzyl viologen agar under one of the electrodes in a N_2 atmosphere, an initial current density of 1 $\mu amp/cm^2$ was usually reached within 5½ to 6 hours and maintained for 8 to 9 hours with a gradual decrease to a negligible current density after 2 day. The electrode with the bacterial cells underneath it (cathode) always showed a positive polarity and the other electrode (no cells) a negative polarity in accordance with the theory, using a standard dry cell as reference. The internal resistance through which the current passed in the voltmeter at the scale setting used was 316 ohms.

Using the value for current density of 1 $\mu amp/cm^2$ and the equivalent corrosion rate[9] for iron of 1 mdd (milligram per dm^2 per day) = 4.0 x 10^{-7} amp/cm^2, the corrosion rate for the cathodic depolarization of steel by a very heavy concentration of resting (non-growing) cells is about 2.5 mdd or 0.00046 inch per year (ipy) by the formula:

$$mdd = ipy \times 696 \times density$$

and the density of 1010 steel as 7.85. According to Uhlig,[8] metals that show a corrosion rate of <0.005 ipy have a "good corrosion resistance to the extent that they are suitable for critical parts, e.g., valve seats, pump shafts, springs, etc."
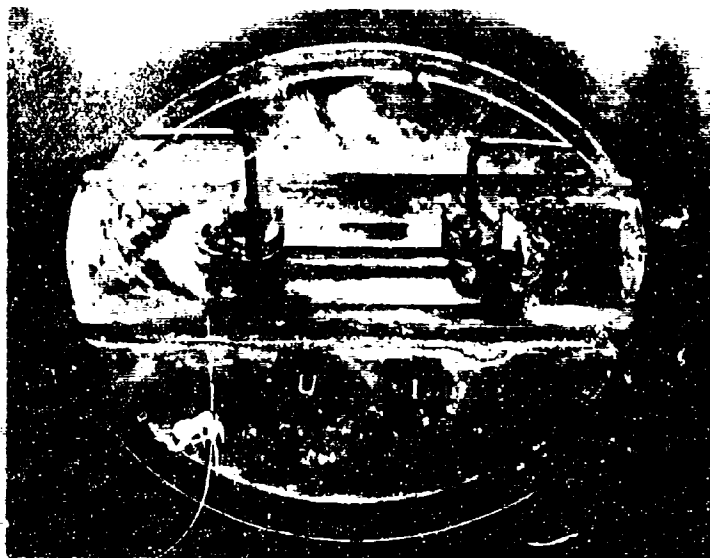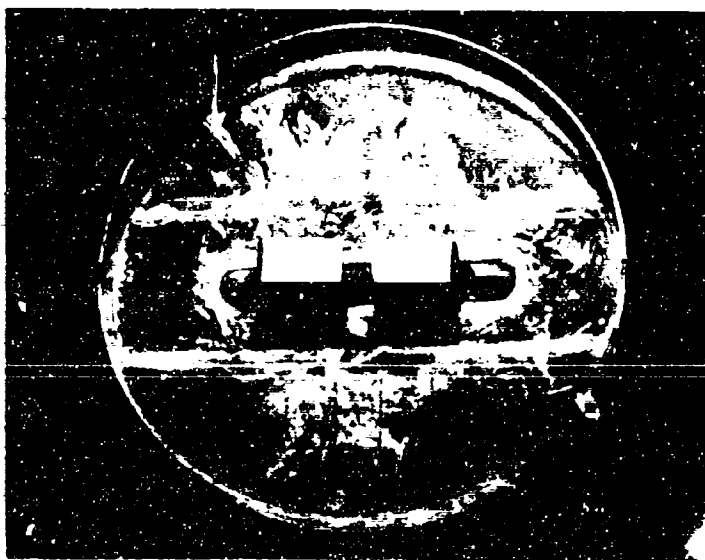
Figure 5. Electrode Holder.
a. Top view.
b. Bottom view.

In nature it is probably doubtful that such a high concentration of cells (exact determinations of cell concentrations will be made) next to a metal surface ever occurs, so that the value of 0.00046 ipy is probably a maximum value.

It thus appears that the mechanism proposed by the theory does indeed operate but that the amount of corrosion brought about by this mechanism is too small to account for the extensive corrosion by sulfate reducers (Desulfovibrio).

Using the techniques previously mentioned, it is possible to study the resistance or susceptibility of other ferrous and nonferrous alloys to cathodic depolarization, the "aggressiveness" of other strains of sulfate reducers and other bacteria possessing active hydrogenase systems in bringing about corrosion by this mechanism, and other environmental factors (viz. pH, influence of other hydrogen donors, electron acceptors, etc.).

For example, it has been demonstrated that the strain of Desulfovibrio used in the studies just reported, as well as a strain isolated from a corrosion pit in an aluminum alloy tank, are able to cathodically depolarize aluminum alloys 2024-T3 and 7075-T6. Metals more noble than iron and aluminum in the electromotive series, such as tin, zinc, and lead, appear to be resistant to this type of attack. This may be due to the toxic effects of these metals and their ions on the hydrogen or other electron transport systems in the cells.

In most of the experiments on corrosion by sulfate reducers reported in the literature, sodium lactate or some other hydrogen donor is used in the medium. Preliminary evidence has been obtained to indicate that these added hydrogen donors decrease corrosion by cathodic depolarization by competing with the hydrogen on the metal surface for the cell hydrogenase system.

Methylene blue can also be used as an electron acceptor instead of benzyl viologen, but the color of the partially reduced dye under the anode interferes with the color produced by the iron reagents. Phosphate buffer appears to be unsatisfactory because it reacts with the iron at the surface to form a gray-blue film of insoluble ferrous phosphate that prevents its detection in the agar.

The technique can easily be made quantitative by measuring the total quantity of cathodic depolarization current in coulombs (using a sensitive coulometer or integrating the area under the current curve) or by the iron concentration in the agar using standard chemical techniques. The two methods should be in agreement (Faraday's Law) if proper controls are used.

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13. ABSTRACT

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